REMARKS

The above amendments to the above-captioned application along with the following remarks are being submitted as a full and complete response to the Official Action dated December 23, 2004.

In view of the above amendments and the following remarks, the Examiner is respectfully requested to give due reconsideration to this application, to indicate the allowability of the claims, and to pass this case to issue.

Status of the Claims

Claims 1-4 and 6-14 are under consideration in this application. Claims 1, 3-4 and 11-12 are being amended, as set forth above, in order to more particularly define and distinctly claim Applicants' invention. Applicants hereby submit that no new matter is being introduced into the application through the submission of this response.

Prior Art Rejections

Under 35 U.S.C. § 102(b), claims 1 - 4, 6 - 9, 11, 12 and 14 were again rejected as being anticipated by WO 98/28440 to Nyren (hereinafter "Nyren"), and claims 1, 3, 4, 6, 7 and 9 were rejected as being anticipated by the article of Nordstrom et al (Analytical Biochemistry (2000) 282:186-193). (hereinafter "Nordstrom"). Under 35 U.S.C. § 103(a), claims 10 and 13 were rejected as being unpatentable over Nyren in view of the article of Ishikawa et al (Human Immunology (1995) 42:315-318, hereinafter "Ishikawa"). These rejections have been carefully considered, but are again most respectfully traversed.

The present invention as now recited in claim 1 is directed to a method of analysis of DNA sequence, comprising the steps of: treating a solution containing a nucleic acid substrate for a complementary strand extension reaction by degrading, using pyrophosphatase, pyrophosphoric acid contained in the solution, and/or degrading, using apyrase, adenosine 5'–triphosphate contained in the solution; removing or inactivating the pyrophosphatase and/or the apyrase in the solution after the pretreating step; mixing the solution with a DNA primer, a target nucleic acid and a reagent for the extension reaction on the DNA primer after the step of removing or inactivating; conducting the extension reaction on the DNA primer hybridized to the target nucleic acid; and detecting pyrophosphoric acid generated by the extension reaction after the removing or inactivating step, wherein the solution does not contain the DNA primer, the target acid and the reagent.

The present invention as recited in claim 3 is now directed to a method of analysis of DNA sequence, comprising steps of: adding pyrophosphatase and/or apyrase to one or more solutions each containing a different deoxynucleotide, or an analogue of the deoxynucleotide and then thereby degrading pyrophosphoric acid or adenosine 5'– triphosphate, respectively, contained in the solutions; removing or inactivating the pyrophosphates and/or the apyrase in the solution after the step of degrading after the adding step; mixing the one or more solutions, a DNA primer, a target nucleic acid and a reagent for extension reaction of the DNA primer after the step of removing or inactivating; and extending the DNA primer hybridized to the target nucleic acid, by using DNA polymerase and at least one of the solutions; and detecting pyrophosphoric acid generated during an extension reaction by chemiluminescence-reaction after the removing or inactivating step, wherein the one or more solutions does not contain the DNA primer, the target acid and the reagent.

In addition, according to claim 4, the present invention is directed to a method of analysis of DNA sequence comprising steps of: adding pyrophosphatase to one or more solutions each containing a different deoxynucleotide, or an analogue of the deoxynucleotide and then thereby degrading pyrophosphoric acid contained in the solutions; removing or inactivating the pyrophosphates in the solutions after the step of degrading after the adding step; mixing the one or more solutions, a DNA primer, a target nucleic acid and a reagent for an extension reaction of the DNA primer after the step of removing or inactivating the pyrophosphatase; extending the DNA primer hybridized to the target nucleic acid, by using DNA polymerase and at least one of the solutions and converting pyrophosphoric acid, generated during the extension reaction, into adenosine 5'-triphosphate in presence of adenosine 5'-phosphosulfate and ATP sulfurylase; and detecting luminescence caused by chemiluminescence-reaction using the adenosine 5'-triphosphate, a luminescence-enzyme and a luminescence substrate after the removing or inactivating step, wherein the one or more solutions does not contain the DNA primer, the target acid and the reagent.

Further, as recited in claim 11, the present invention is directed to a method of analysis of DNA sequence, comprising steps of: a first step of adding pyrophosphatase to each of a solution containing deoxyadenosine 5'-α-thiotriphosphate, a solution containing deoxythymidine 5'-triphosphate, a solution containing deoxyguanosine 5'-triphosphate and a solution containing deoxycytidine 5'-triphosphate, and then thereby degrading pyrophosphoric acid contained in each of the solutions; a second step of removing or inactivating the pyrophosphatase in each of the solutions after the first step; a third step of mixing the one or

more solutions, a DNA primer, a target nucleic acid and a reagent for extension reaction of the DNA primer after the second step; a fourth step of extending the DNA primer hybridized to the target nucleic acid, by using DNA polymerase and at least one of the solutions obtained in said second step, converting pyrophosphoric acid generated during the extension reaction into adenosine 5'-triphosphate in presence of adenosine 5' phosphosulfate and ATP sulfurylase; and a fifth step of detecting luminescence caused by chemiluminescence-reaction using the adenosine 5'-triphosphate, lusiferase and luciferin after the second step, wherein each of the solutions does not contain the DNA primer, the target acid and the reagent.

Even more, the present invention as recited in claim 12 is directed to a method of analysis of DNA sequence, comprising steps of: a first step of adding pyrophosphatase to a solution containing deoxyadenosine 5'-α-thiotriphosphate, deoxythymidine 5'-triphosphate, deoxyguanosine 5'-triphosphate and deoxycytidine 5'-triphosphate, thereby degrading the pyrophosphoric acid contained in the solution; a second step of removing or inactivating the pyrophosphatase in each of the solutions after the first step; a third step of mixing the one or more solutions, a DNA primer, a target nucleic acid and a reagent for an extension reaction of the DNA primer after the second step, and a fourth step of extending the DNA primer hybridized to the target nucleic acid, by using DNA polymerase and at least one of the solutions obtained in said second step, converting pyrophosphoric acid, generated during the extension reaction, into adenosine 5'-triphosphate in presence of adenosine 5' phosphosulfate and ATP sulfurylase; and a fifth step of detecting luminescence caused by chemiluminescence-reaction using the adenosine 5' triphosphate, lusiferase and luciferin after the second step, wherein each of the solutions does not contain the DNA primer, the target acid and the reagent.

Among the main features of the present invention as recited in at least the above-noted claims, the method of the invention incorporates "treating a solution containing a nucleic acid substrate for complementary strand extension reaction with degrading, using pyrophosphatase, pyrophosphoric acid contained in the solution", "removing or inactivating the pyrophosphatase and/or the apyrase in the solution after the step of treating," and "mixing the solution, a DNA primer, a target nucleic acid and a reagent for extension reaction of the DNA primer after the step of removing or inactivating", "wherein the solution does not contain the DNA primer, the target acid and the reagent," such as that recited in claim 1.

Applicants will point out that four dNTPs generate PPi (pyrophosphoric acid) by thermal degradation or the like and thus generate the largest noise signal (p. 27, line 16; p. 19,

lines 3-7; p. 20, lines 9-15). Depending on the reagent supplied by different companies with different manufacturing methods, different lots and different storage conditions, the amounts of PPi contained as impurities in four dNTPs or analogies differ (p. 27, lines 11-18), thus creating different amount of background noise. In order to reduce the noise caused by a large amount of PPi generated by thermal degradation of the nucleic acid substrate (dNTPs), the present invention incorporates the step of treating a solution containing a nucleic acid substrate for complementary strand extension reaction with degrading, using pyrophosphatase, pyrophosphoric acid contained in the solution, wherein the solution does not contain the DNA primer, the target acid and the reagent, such as in claim 1.

Applicants will further point out that pyrophosphatase breaks the phosphate bond which is exists not only in PPi, but also in dNTP. At the initial stage of treating using pyrophosphatase, the amount of dNTP is much more than the amount of PPi and the amount of degraded dNTP can be negligible. However, after the degradation of PPi, pyrophosphatase degrades only dNTP. That is, pyrophosphatase will degrade too much dNTP, if it remains in the solution for a long time, thereby resulting in the loss of dNTP and consequently in low detection sensitivity. To resolve this matter, the present invention incorporates removing or inactivating the pyrophosphatase and/or the apyrase in the solution after the step of treating (see for example, claim 1). Through this combination of steps or features, among others, the present invention can reduce the cause of noise while avoiding excess dNTP degradation, thereby achieving a sufficient sensitivity detection level for chemiluminescence reaction.

Applicants respectfully contend that none of the cited prior art references teaches or suggests the combination of steps or features as noted above, as recited for present the invention.

In contrast, the Office Action recites that Nyren's description of "In carrying out the method of the invention, any possible . . ." expressly teaches adding pyrophosphatase to the reagent solutions, which will occur prior to any reaction occurring. At best, this step corresponds to step 106 of Fig.1 of the present application. Nyren does not show or suggest any step of removing or inactivating the pyrophosphatase and/or the apyrase in the solution after the step of treating, such as that recited in claim 1.

Nyren merely discloses that "any possible contamination of the reagents e.g. the NTP solutions, by PPi is undesirable and may readily be avoided by including a pyrophosphatase, preferably in low amounts, in the reagent solutions" (see p. 19, lines 2 -6). However, as described above, pyrophosphatase breaks the phosphate bond not only in PPi, but also in

dNTP. Thus, if the pyrophosphatase remains in the NTP solution even in low amounts, it will continually degrade the NTP, thus inevitably resulting in excessive degradation of NTP and low detection sensitivity. Further, to the extent that Nyren describes adding a pyrophosphatase in low amounts in the reagent solutions, or even allowing a pyrophosphatase to remain in low amounts, this reference falls far short of disclosing or even suggesting any step of removing or inactivating the pyrophosphatase and/or the apyrase in the solution after the step of treating. Therefore, Nyren cannot and does not show or suggest at least the above features of the present invention, nor the advantages achieved through those features of the present invention.

On p. 8, line 20 to p. 9, line 3 of the Office Action, the Examiner argues that Nyren teaches the use of apyrase at page 5 and that the same enzyme is used in both methods and function in the same way in both methods. However, contrary to that assertion, Nyren in actuality describes that "the nucleotide-degrading enzyme or enzymes are simply included in the polymerase reaction mix, and a sufficient time is allowed between each successive nucleotide addition for degradation of substantially most of the unincorporated nucleotides" (see p. 5, lines 24-28), and that "enzyme(s) may be added after nucleotide incorporation (i.e., chain extension) has taken place, and then, when the incorporated nucleotides are hydrolyzed, the immobilized enzyme may be removed from the reaction mixture (e.g., it may be withdrawn or captured, such as magnetically in the case of magnetic beads), before the next nucleotide is added. The procedure may then be repeated to sequence more bases" (see p. 6, lines 17-25).

There is no such corresponding step in the present invention. At best, this step would occur in the loop of step 112-114 if done at all in the implementation of the present invention. Then, Nyren does not show any step of "removing or inactivating the pyrophosphatase and/or the apyrase in the solution after the step of treating," wherein "the solution" is a solution containing a nucleic acid substrate for complementary strand extension reaction and that does not contain the DNA primer, the target acid and the reagent. The solution in the present invention is quite different from Nyren's polymerase reaction mix. Therefore, Nyren's disclosure of a nucleotide degrading enzyme cannot and does not meet or apply to any claim limitation of the present invention.

Regarding Nordstrom, the Examiner pointed on p. 9, lines 4-7 of the Office Action that this reference treats a solution with pyrophosphatase prior to the remaining steps. Nordstrom describes that the PCR amplified product was incubated with apyrase or some kinds of pyrophosphatase and after the enzymatic treatment, the sample was heated to 94-100°C before the sequencing primer was added ("Enzymatic preparation of dsDNA templates" in p. 187).

These kinds of pyrophosphatase are irreversibly inactivated at high temperature (p. 192, lines 20-22). There is no such corresponding step in the present invention, and thus this step from Nordstrom is irrelevant to the present invention. Rather, Nordstrom fails to show or suggest, among other features, the step of removing or inactivating the pyrophosphatase and/or the apyrase in the solution after the step of treating, wherein the solution is "a solution containing a nucleic acid substrate for complementary strand extension reaction" that does not contain the DNA primer, the target acid and the reagent. At least this feature is distinguishably different from Nordstrom's "PCR amplified products". Therefore, Nordstrom's use of pyrophosphatase cannot and does not meet or apply to any claim limitation of the present invention.

Overall, the reference to Nordstrom falls far short of anticipating each and every feature of the present invention as claimed.

With respect to the rejection under 35 USC §103(a), as explained above, Nyren by itself fails to anticipate each and every feature of the claimed invention. Further, Applicants will contend that this reference by itself also fails to provide any disclosure or suggestion that would render each and every feature of the claimed invention obvious to one of skill in the art. The secondary reference to Ishikawa merely describes the use of primers for detecting a single base difference between A2 alleles and other HLA-A alleles, having one extra mismatch at the second position from its 3'-end (See Abstract). Ishikawa fails to provide any teaching or suggestion that would make up for the deficiencies in Nyren as described above, such that their combination could render the features of the present invention obvious. In other words, even if these two references were combined, that combination would still fall short of embodying all the claimed features of the present invention. Thus, the present invention as claimed cannot be rendered obvious in view of Nyren and Ishikawa.

Conclusion

In view of all the above, clear and distinct differences as discussed exist between the present invention as now claimed and the prior art references upon which the rejections in the Office Action rely, Applicants respectfully contend that the prior art references cannot anticipate the present invention or render the present invention obvious. Rather, the present invention as a whole is distinguishable, and thereby allowable over the prior art.

Favorable reconsideration of this application is respectfully solicited. Should there be any outstanding issues requiring discussion that would further the prosecution and allowance of the

above-captioned application, the Examiner is invited to contact the Applicants' undersigned representative at the address and phone number indicated below.

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